Contact Sensitizers Induce Keratinocytes to Release Epitopes

Tools for *In Vitro* Tests and Implications for Autoimmunity

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Akademisk avhandling för avläggande av filosofie doktorsexamen i naturvetenskap inriktning kemi som, med medgivande av Institutionen för Kemi, Göteborgs Universitet, kommer att försvaras offentligt fredagen den 9 december kl 9.00 i sal KC, Kemigården 4, Göteborgs Universitet och Chalmers Tekniska Högskola. Avhandlingen kommer att försvaras på engelska.

Fakultetsopponent är Professor Thomas Rustemeyer, Department of Dermatology, VU University Medical Center, Amsterdam, Nederländerna.

ABSTRACT

Contact allergy and its clinical manifestation, allergic contact dermatitis, affect approximately 20 % of the population in the Western world. It is caused by small reactive chemical compounds, called haptens. Haptens are thought to react with proteins in the skin and create immunogenic hapten-protein complexes. However, little is known about which proteins are covalently modified by haptens.

In this thesis, using caged bromobimanes as chemical probes, the basal keratinocytes and their cytoskeletal keratin intermediate filaments were shown to be hapten targets in *ex vivo* human skin. Furthermore, the first exact hapten target site detected against the backdrop of the entire proteome of the skin was presented.

Human cultured keratinocytes were found to release hapten modified keratins, as well as other possibly modified proteins, in blebs (plasma membrane vesicles) when exposed to haptens. The exact hapten target site found in skin samples were again found in blebs released by living cultured keratinocytes, indicating that the finding in *ex vivo* skin has *in vivo* relevance. Since the blebs contain hapten modified proteins, the hypothesis that blebs may play a role in sensitization in contact allergy is proposed.

In response to the European Union ban of testing cosmetic products or ingredients of cosmetic products on animals, the blebbing response of keratinocytes was utilized in a pilot study toward developing a new *in vitro* test for assessing the sensitizing potency of chemicals. The results are promising and it is hoped that the bleb response will be able to form the basis of a new, alternative, non-animal based test.

Further investigations of the bleb content revealed that several of the proteins present in blebs are known autoantigens in a variety of autoimmune disorders. Analysis of serum from hapten-exposed mice showed antibodies against a selection of the identified proteins as well as another marker of autoimmunity. However, the clinical relevance of the detected autoantibodies is unknown.

As keratins were found to be hapten targets, potential antibody responses against keratins in serum of hapten-exposed mice were analyzed in the final part of this thesis. The positive identification of antikeratin antibodies supports the previous results obtained from skin and cultured cells. Epitope mapping along the primary keratin sequences revealed different antibody binding patterns for different hapten exposures, thus providing new insights in which part of the protein that trigger a specific immune response.

In conclusion, the work presented in this thesis gives new, exciting insights into the mechanisms behind contact allergy as well as new tools for *in vitro* testing. It also demonstrates the power of combining chemistry and biology with high-tech microscopy and proteomic techniques when studying hapten-protein interactions in skin and cultured cells.

Keywords: Contact allergy, Bromobimanes, Caged fluorophores, Hapten targets, Hapten-protein complex, Keratin 5, Keratin 14, Blebs, Keratin bodies, Sensitizing potency, *In Vitro* assay, Alternative Methods, Bleb content, Autoimmunity, Epitope mapping

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